APPLICATIONS OF LIPOSOMES IN IMMUNOPOTENTIATION*

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LIPOSOMES

Liposomes are membranous vesicles composed predominantly of phospholipids (Bangham, 1972). When dry phospholipids are confronted with an aqueous solution, they undergo a series of rearrangements resulting in the formation of concentric lipid bilayers alternating with aqueous compartments. Upon ultrasonication of these large multilamellar vesicles (MLV, 400-3,500 nm), small unilamellar vesicles (SUV, 20-50 nm) consisting of a single lipid bilayer can be obtained (Huang & Thompson, 1974; Szoka & Papahadjopoulos, 1980). Methods have also been developed for the preparation of large unilamellar vesicles (LUV, 200-1,000 nm) by the fusion of SUV (Papahadjopoulos et al., 1975), by the vaporization of an ether solution of lipid (Deamer & Bangham, 1976), and by the removal of detergent from dissolved lipids (Milsmann, Schweidener & Weder, 1978; Enoch & Strittmatter, 1979). The physical structures of liposomes can be modified in terms of their lipid composition, size, surface charge, and membrane lamellar arrangements. During the process of liposome formation, water-soluble solutes may be entrapped within the aqueous compartments and hydrophobic substances may be incorporated in the lipid bilayers of the vesicle. The attractiveness in the application of liposomes as a carrier system resides in the compatibility of the constituent components with the body system. Being made up of naturally occurring lipid materials, e.g., lecithin and cholesterol, liposomes are biodegradable, relatively non-toxic, and very feebly immunogenic by themselves (Adams et al., 1977; Gregoriadis, 1978; Hart et al., 1981). Liposomes have been used as couriers for a wide variety of materials including enzymes (Gregoriadis & Ryman, 1972; Weissmann et al., 1978), drugs (Rahman et al., 1974; Kimelberg et al., 1976; Fendler & Romero, 1977; Alving et al., 1978; Kaye et al., 1981; Gregoriadis, 1981), protein antigens (Allison & Gregoriadis, 1974; Heath, Edwards

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& Ryman, 1976; Van Rooijen & Van Nieuwmegen, 1977; Shek & Sabiston, 1982a), and hormones (Patel & Ryman, 1976; Shaw et al., 1976). Among the many potential applications of liposomes in therapeutic and preventive medicine (Ryman & Tyrrell, 1980; Gregoriadis, 1981), the first to be realized may be the use of liposomes as immunopotentiating carriers.

POTENTIATION OF THE ANTIBODY RESPONSE TO LIPOSOME-ASSOCIATED PROTEIN ANTIGENS

The effectiveness of the association of a protein antigen to liposomes in enhancing the anti-protein antibody response was originally described by Allison and Gregoriadis (1974). In contrast to their initial anticipation that the entrapment of an antigen within liposomal vesicles might render it non-immunogenic, it was found that liposome-encapsulated diphtheria toxoid elicited a much higher antibody response against diphtheria than that induced by the free toxoid. The demonstration of the adjuvant effect of liposomes has subsequently been extended to other protein antigens in terms of enhanced circulating antibody titers (Heath et al., 1976; Van Rooijen & Van Nieuwmegen, 1977; Six et al., 1980) and elevated antibody-forming cell response (Shek & Sabiston, 1982a). Unlike other potent immunoadjuvants, e.g., complete Freund's adjuvant, which are unacceptable for human use because of undesirable necrotic reactions at the site of injection, liposome-encapsulated antigens do not induce local granuloma formation (Allison & Gregoriadis, 1976). Another advantage of using liposomes as an immunostimulator is its apparent ineffectiveness in provoking delayed hypersensitivity reactions to the encapsulated antigen (Gregoriadis & Allison, 1974). Furthermore, the lipids used in the synthesis of the commonly used liposomal vesicles are not foreign to the body and no apparent ill effects have been found in injected human subjects (Allison & Gregoriadis, 1976; Richardson et al., 1979; Belchetz et al., 1977). Although liposomal vesicles may prove to be effective and safe vaccine carriers, the critical physicochemical factors of these vesicles in stimulating an optimal response are ill-defined and the cellular mechanisms mediating their immunopotentiating effects have not been established.

Lipid Composition

A variety of lipid constituents has been used in the preparation of liposomes for immunopotentiation. The basic backbone of the liposome is its phospholipid component and the most commonly used phospholipid in liposome preparations is phosphatidylcholine. Some examples of phosphatidylcholine-containing liposomes that have been found effective in immunopotentiation are

TABLE I

LIPID COMPOSITION OF LIPOSOMES EFFECTIVE IN POTENTIATING THE ANTIBODY RESPONSE TO SOME PROTEIN ANTIGENS

Protein Antigens	Lipid composition	References
Diphtheria toxoid	7PC:2CSL:1DCP	Allison & Gregoriadis, 1974
	7PC:2CSL:1PA	Allison & Gregoriadis, 1976
Bovine serum albumin	9PC:1DCP	Heath et al., 1976
	7PC:2CSL:1PA	Shek & Sabiston, 1982a
Human serum albumin	7PC:2CSL:1PA	Van Rooijen & Van Nieuwmegen, 1977
Bovine gamma globulin	7PC:2CSL:1PA	Van Rooijen & Van Nieuwmegen, 1980
Hepatitis B surface antigen	7PC:2CSL:1DCP	Gregoriadis & Manesis, 1980
Adenovirus protein antigen	10DPPC:7.5CSL:1PA	Six et al., 1980
Gross-virus cell surface antigen	7PC:2CSL:1DCP	Gerlier et al., 1980

PC, phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; CSL, cholesterol; DCP, dicetyl phosphate; PA, phosphatidic acid.

listed in Table I. In most studies, the molar ratio of the lipids used is empirically determined. Nevertheless, liposomes of varied lipid composition and surface charge are capable of enhancing the antibody response to a number of protein antigens. The incorporation of cholesterol in the liposomal membrane enhances the structural integrity of the lipid bilayer (Finkelstein & Weissmann, 1979) and a high cholesterol/phospholipid ratio reduces the permeability of the vesicle (Papahadjopoulos et al., 1972). Although a relatively high cholesterol content was reported to reduce the antibody response to the entrapped antigen (Heath et al., 1976), the critical role of cholesterol in supporting the adjuvanticity of liposomes has not been analyzed. It has been reported that the immunogenicity of hapten-substituted liposomes is related to the lipid composition and the phase-transition temperatures of the phospholipids used (Yasuda et al., 1977; Van Houte et al., 1981); however, similar studies have not been conducted for liposome-associated protein antigens. It is of interest to note that haptenated liposomes prepared from glycerophospholipid are much less efficient than those prepared from sphingophospholipid in potentiating the hapten-specific antibody response (Yasuda et al., 1977). In contrast, phosphatidylcholine liposomes are more potent than sphingomyelin liposomes in promoting antibody formation to liposome-associated protein antigens (Heath et al., 1976).

The introduction of surface charge to liposomal vesicles may result in different potentiating effects. Thus, positively-charged liposomes were found ineffective in promoting the anti-diphtheria antibody response (Allison & Gregoriadis, 1974) but were proficient in enhancing the antibody response to serum albumin (Heath et al., 1976). The incorporation of a charged lipid results in the bilayers repelling one another and this increases the entrapped aqueous space (Bangham et al., 1967). The extent to which charged vesicles interact electrostatically with proteins varies, depending on the isoelectric point of the protein involved. Thus, surface charge characteristics not only affect the extent of protein entrapment but also influence the non-covalent interactions of the lipid bilayers with the protein antigen. The nature of association between the vesicle and the antigen may influence the resulting antigenicity of the liposome-associated protein.

MLV versus LUV

Most studies conducted to investigate the adjuvant effect of liposomes invariably used MLV as antigen carriers. This may stem from their ease of preparation and their sufficient effectiveness in potentiating the immunogenicity of the antigen being examined. In order to determine whether the lamellar structure of liposomal carriers may influence the magnitude of the provoked antibody response, MLV and LUV of approximately the same size range were used to compare their efficacy in promoting the response to their entrapped antigens. It can be seen from Table II that LUV is much more effective than MLV in potentiating the plaque-forming cell (PFC) response to the entrapped bovine serum albumin (BSA) in mice. The reason for this difference is not known. However, LUV has been shown to have a larger captured volume than MLV resulting in a higher efficiency of encapsulation (Papahadjopoulos et al., 1980). Furthermore, the uptake of entrapped antigen by phagocytic cells is proportional to the captured volume of the vesicle (Poste et al, 1980). Thus, in all likelihood, the entrapped antigen of LUV should be more readily available to the interacting cell, e.g., a macrophage, since the vesicle is enclosed by only a single lipid bilayer. Alternatively, the difference in immunoenhancing capacity between MLV and LUV may be related to different patterns of homing to the target lymphoid organ.

TABLE II

ANTI-BSA PFC RESPONSE ELICITED BY MLV- AND LUV-ASSOCIATED BSA IN A/J MICE

Antigen[a]	Number of Mice	BSA-specific IgG PFC/spleen[b]	p[c]
MLV-BSA	8	59,200 ± 7,600	
LUV-BSA	8	139,000 ± 15,000	< 0.0005

[a] BSA was entrapped in MLV (330 ± 70 nm) or LUV (240 ± 50 nm) composed of dimyristoyl phosphatidylcholine, cholesterol and dicetyl phosphate in a molar ratio of 7:2:1. Mice were given 2 identical intraperitoneal injections, 3 weeks apart, of each antigen preparation containing 30 µg of BSA protein.

[b] Splenic BSA-specific PFC response was assayed 4 days after the second injection of antigen.

[c] p value (Student's t-test) comparing the PFC response of the two groups.

Site of Association

One crucial parameter which determines the effectiveness of liposomes in potentiating the antibody response to an antigen is the physical association between the two entities. Although liposome-associated BSA consistently elicited an enhanced anti-BSA antibody response, the separate but simultaneous injection of liposomes and the free BSA (fBSA) failed to elicit an appreciable response (Fig. 1). Thus, a direct physical association of the antigen with the liposomal vesicle is imperative for successful immunopotentiation to occur. Although physical linkage between the antigen and the liposome is essential, opposite views have been expressed regarding the importance of the actual site of association. Using different liposome-antigen systems, suggestions were put forward favouring either internal entrapment (Six et al., 1980) or surface adsorption of the antigen (Van Rooijen & Van Nieuwmegen, 1980) as being essential in liposome-mediated enhancement. In order to investigate this apparent discrepancy, the relative immunogenicity of a protein antigen associated with liposomes on the surface or entrapped internally was compared under one experimental system. Different preparations of the appropriate liposome-associated BSA antigens used are shown in Fig. 2. It was found that animals immunized with liposomes, containing both entrapped and surface-adsorbed BSA, $(LSM\text{-}BSA)_{e+a}$, gave a BSA-specific PFC response comparable to that elicited by animals immunized with trypsinized $(LSM\text{-}BSA)_{e+a}$ which contained mainly entrapped BSA (Fig. 3). In contrast, the PFC response elicited by animals immunized with liposomes containing only BSA adsorbed on the membrane surface, $(LSM\text{-}BSA)_{a}$, was significantly less than that of

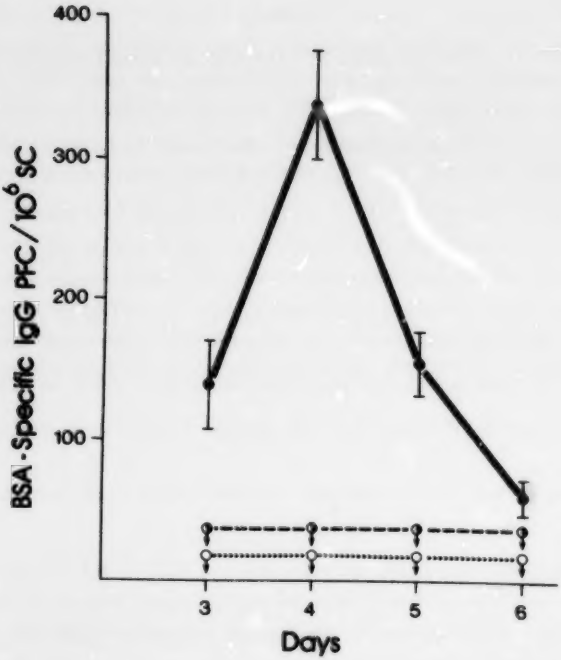

Figure 1. Potentiation of the BSA-specific PFC response by LSM-BSA. A/J mice were given two intraperitoneal injections of 50 μg of either LSM-BSA (●), fBSA (○), or fBSA together with empty LSM (⊕), 2 weeks apart. Spleen cells of the immunized animals were harvested for the PFC assay 3-6 days after the second injection of antigen. Each point represents the mean response ± SEM of seven to ten animals. The inverted arrow indicates a mean response of less than 10 PFC per 10^6 spleen cells. (From Shek & Sabiston, 1982a).

the above two groups. Finally, animals injected with trypsinized $(LSM\text{-}BSA)_a$ only elicited a marginally detectable PFC response. These results demonstrate that liposomes are effective in potentiating the antibody response to the associated protein antigen irrespective of the site of association, although the entrapped antigen appears to be more immunogenic.

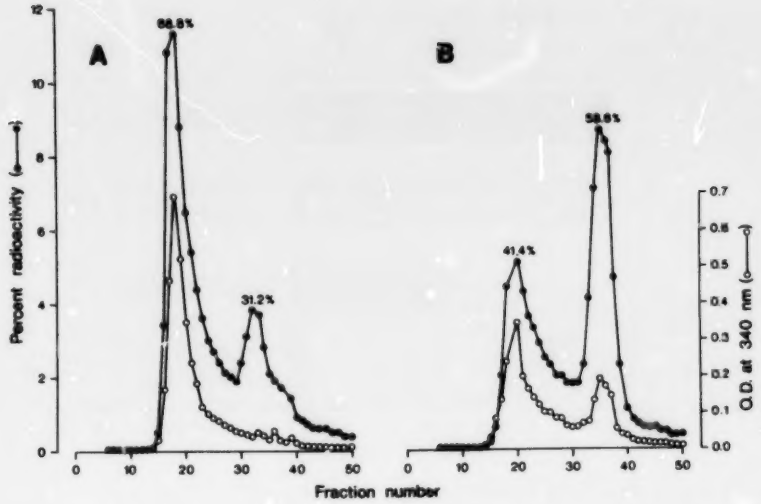

Figure 2a. Sepharose-4B chromatography of A: $(LSM\text{-}BSA)_{e+a}$ and B: trypsinized $(LSM\text{-}BSA)_{e+a}$. A dried lipid film, composed of phosphatidylcholine, cholesterol, and phosphatidic acid in a molar ratio of 7:2:1, was resuspended in a protein solution containing unlabelled BSA (12 mg/ml) and ^{125}I-labelled BSA (14.2 Ci/mmol). After an incubation period of 2 hr at room temperature, the liposomal suspension was sonicated (100 watts, 30 sec) and most of the protein molecules not associated with the liposome were removed by centrifugation at 100,000 g for 30 min. The resulting liposomal pellet which contained entrapped and surface adsorbed BSA molecules, $(LSM\text{-}BSA)_{e+a}$, was resuspended and divided into two equal aliquots. One aliquot was passed through a Sepharose-4B column (1.5 x 30 cm) equilibrated with 0.15M phosphate-buffered saline, pH 7.2 (panel A) and the other aliquot was treated with trypsin before its passage through the same column (panel B). Fractions of 2 ml were collected and protein recovery in each fraction was expressed as a percentage of the recovered radioactivity. The percentage figure indicated at the top of each peak represents the summation of the total radioactivity of the fractions of that peak.

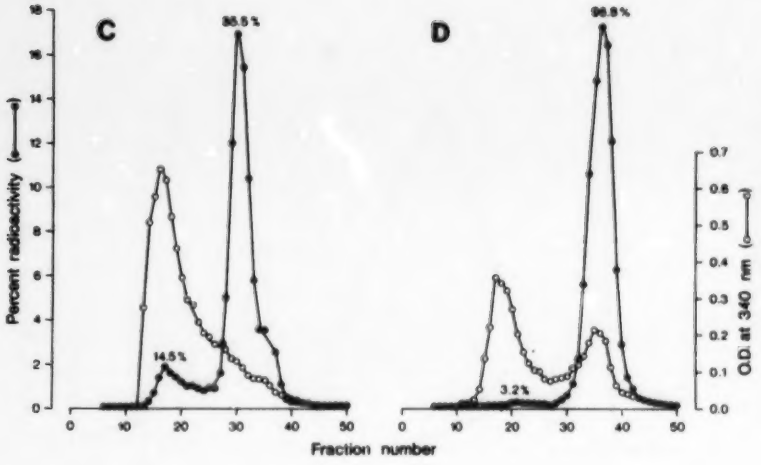

Figure 2b. Sepharose-4B chromatography of C: $(LSM\text{-}BSA)_a$, and D: trypsinized $(LSM\text{-}BSA)_a$. Preformed empty liposomes, composed of phosphatidylcholine, cholesterol, and phosphatidic acid in a molar ratio of 7:2:1, were mixed with a protein solution containing unlabelled BSA (12 mg/ml) and ^{125}I-labelled BSA (14.2 Ci/mmol). After an incubation period of 2 hr at room temperature, the liposomal suspension was sonicated (100 watts, 30 sec) and most of the protein molecules not associated with the liposomes were removed by centrifugation at 100,000 g for 30 min. The resulting liposomal pellet which contained surface-adsorbed BSA molecules, $(LSM\text{-}BSA)_a$, was resuspended and divided into two equal aliquots. One aliquot was passed through a Sepharose-4B column (1.5 x 30 cm) equilibrated with 0.15M phosphate-buffered saline, pH 7.2 (panel C) and the other aliquot was treated with trypsin before its passage through the same column (panel D). Fractions were collected and monitored as described in Fig. 2a (From Shek & Sabiston, 1982b).

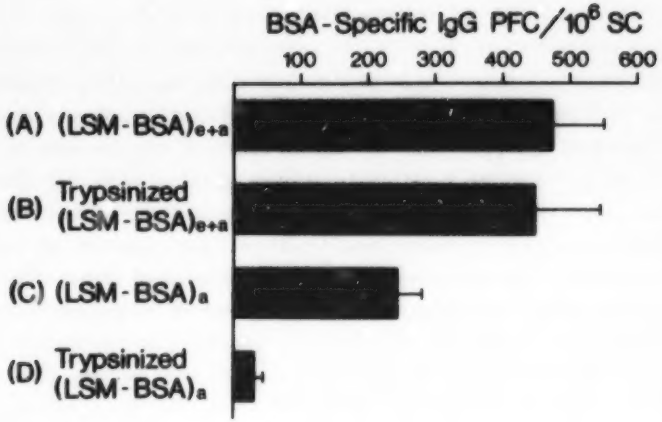

Figure 3. BSA-specific PFC response to immunizations with 4 preparations of liposome-associated BSA antigen. Different groups of A/J mice were given two intraperitoneal injections, 0.5 mg per injection, of one of the LSM-BSA preparations, 3 weeks apart. Each LSM-BSA preparation was obtained from the pooled fractions of the excluded peak of the corresponding chromatographic run shown in Fig. 2. Immunized animals were killed 4 days after the second injection of antigen and their spleen cells were harvested for the PFC assay. Comparison of the mean PFC response by Student's t-test between groups: A and B (not significant); A and C ($p < 0.01$); C and D ($p < 0.001$) (From Shek & Sabiston, 1982b).

Route of Administration

The administration of liposome-associated antigens via the intraperitoneal, subcutaneous, intramuscular, and intravenous routes has been found to be effective in enhancing the antibody response. Although the intravenous route was considered to be a poor way of immunizing mice with liposome-entrapped antigen (Tyrrell et al., 1976), consistent immunoenhancement was obtained in rabbits via this route (Van Rooijen & Van Nieuwmegen, 1977). Immunization of mice by subcutaneous injections with liposome-associated albumin elicited a higher antibody titer than via the intravenous route (Heath et al., 1976), but the reverse appears to be true if the entrapped antigen is diphtheria toxoid (Allison & Gregoriadis, 1976). Most likely, the route of antigen administration is not the only parameter which determines the magnitude of immunoenhancement. Other factors, such as the physicochemical properties of the liposomal carrier, the biochemical nature of the protein antigen used, the mode of liposome-antigen association, and the dose of antigen employed, may also contribute to modulating the immunostimulatory effect.

T-Cell Dependency

The antibody response to most protein antigens, e.g., BSA, requires the participation of thymus-derived (T) cells (Taylor, 1969; Allison & Davis, 1971). Since it has been reported that the hapten-specific PFC response to haptenated liposomes does not require T-cell help (Yasuda et al., 1979; Van Houte et al., 1979), the question arises whether liposome-encapsulated protein antigens can become T-independent. It was found that the immunization of animals with liposome-associated BSA failed to induce either a primary (Table III) or secondary (Shek & Sabiston, 1982a) anti-BSA PFC response in congenitally athymic nude

TABLE III

COMPARISON OF ANTIGEN-SPECIFIC PRIMARY PFC RESPONSE TO LSM-BSA AND TKS AMONG NORMAL BALB/c MICE, HOMOZYGOUS ATHYMIC NUDE (Nu/Nu) MICE, AND HETEROZYGOUS (Nu/+) LITTERMATES

Strain[a]	Antigen	PFC Response	p[f]
		BSA-specific PFC/spleen[c]	
BALB/c (+/+)	LSM-BSA[b]	5,570 ± 640	
BALB/c (Nu/+)	LSM-BSA	4,930 ± 490	n.s.
BALB/c (Nu/Nu)	LSM-BSA	30 ± 10	< 0.0001
		TNP-specific PFC/spleen[e]	
BALB/c (+/+)	TKS[d]	11,380 ± 590	
BALB/c (Nu/+)	TKS	13,500 ± 650	n.s.
BALB/c (Nu/Nu)	TKS	11,150 ± 920	n.s.

[a] 5 animals in each group.

[b] 50 µg LSM-BSA injected intraperitoneally.

[c] BSA-specific indirect PFC response was assayed 6 days after immunization.

[d] 100 µg TNP-KLH-Sepharose (2,4,6-trinitrophenylated keyhole-limpet-hemocyanin-conjugated Sepharose, a T-cell independent antigen) injected intraperitoneally.

[e] TNP-specific direct PFC was assayed 8 days after immunization.

[f] p value (Student's t-test) in comparison to the corresponding PFC response of normal BALB/c (+/+) animals.

(Nu/Nu) mice. This provides direct evidence demonstrating the obligatory role of T cells in mediating the immunostimulatory effect of liposomes for encapsulated protein antigens. It is possible that entrapped protein antigens are not directly accessible to B cells; whereas, the substitution of haptenic molecules, especially at high epitope densities, on the liposome surface, may facilitate the direct activation of B cells. Another line of observation which implicates thymic involvement in immunopotentiation, mediated by liposome-associated protein antigen, is the capacity of encapsulated antigen to generate a strong immunological memory. It is evident from Fig. 4 that mice previously primed with liposome-associated BSA elicited an intensive secondary PFC response, even when challenged with the weakly immunogenic free BSA. The amplification of an anamnestic IgG response requires the modulation of long-lived recirculating memory T cells (Miller, 1973). Thus, our data appear to suggest that liposome-encapsulated protein antigen is proficient in provoking a thymic-dependent memory.

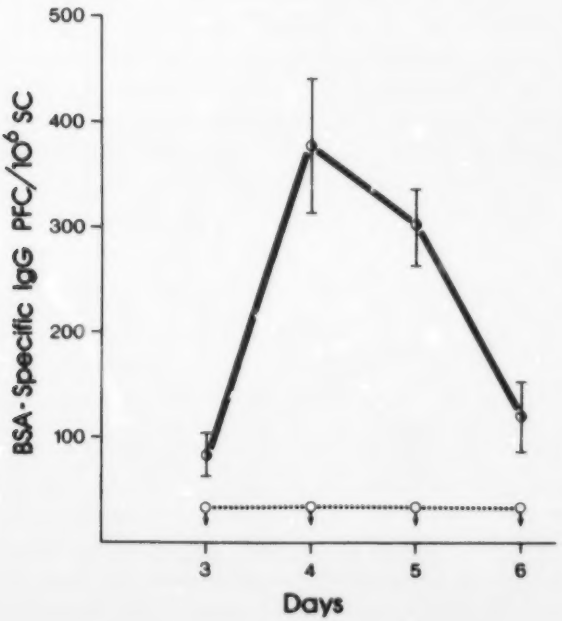

Figure 4. Effectiveness of LSM-BSA in priming animals for a secondary antibody response. A/J mice were divided into two groups and each group was injected intraperitoneally with 50 μg of either LSM-BSA (●), or free BSA (○). After a resting period of 5 weeks, all animals were challenged with 50 μg of free BSA. The spleen cells of the immunized animals were harvested for the PFC assay 3-6 days after antigenic challenge. Each point represents the mean response ± SEM of five to six animals. The inverted arrow indicates a mean response of less than 10 PFC per 10^6 spleen cells (From Shek & Sabiston, 1982a).

Role of Macrophages

In view of the fact that T cells are required in promoting the antibody response to liposome-encapsulated protein antigens, the involvement of an accessory cell, e.g., macrophage, in antigen processing is very likely. In order to determine whether macrophage participation is essential, the effect of the depletion of macrophages on the antibody response to liposome-entrapped proteins was studied. The abrogation of macrophage function was achieved by the *in vivo* administration of carrageenan, a sulfated polygalactose. Carrageenan is cytotoxic for phagocytic cells, but exerts no harmful effect on lymphocytes (Cantanzaro *et al.*, 1971; Ishizaka *et al.*, 1977; Thomson *et al.*, 1979). It is evident from Fig. 5 that the injection of mice with carrageenan suppressed their PFC response to sheep red blood cells (SRBC), a macrophage-dependent T-cell antigen (Mitchell & Miller, 1968; Gorczynski *et al.*, 1971) but had no effect on the response to *E. coli* lipopolysaccharide, a macrophage-independent B-cell antigen (Wong & Herscowitz, 1979). The pretreatment of mice with carrageenan suppressed their PFC response to liposome-entrapped BSA (Table IV) and abolished their circulating anti-BSA antibody titers (Fig. 6). Our results support the view that macrophages play an obligatory role in mediating the antibody response to liposome-associated protein antigens. *In vitro* studies on the immunogenicity of haptenated liposomes also demonstrate the requirement of macrophages for successful induction of a hapten-specific PFC response, but the macrophage requirement can be replaced by the addition of 2-mercaptoethanol (Yasuda *et al.*, 1979) or of a soluble factor released by Concanavalin A-stimulated spleen cells (Humphries, 1981).

Vaccine Carrier

An ideal vaccine carrier is one which is capable of potentiating the immunogenicity of purified vaccine subunits or specific antigenic determinants, without provoking undesirable local and systemic allergic reactions. The carrier itself should be harmless, non-foreign to the body, and easily metabolized. Being composed of naturally-occurring lipids, appropriately prepared liposomes possess most, if not all, of these desirable features. Phosphatidylcholine, the most commonly used phospholipid for liposome preparations, is practically non-immunogenic by itself (Gregoriadis, 1978; Scherphof *et al.*, 1981). The modification of phosphatidylcholine liposomes by the incorporation, in the lipid bilayers, of other adjuvant substances, e.g., lipid A of bacterial lipopolysaccharide, however, can induce the formation of anti-liposome antibodies (Schuster *et al.*, 1979). Thus, the lipid composition of a vaccine carrier should be chosen to

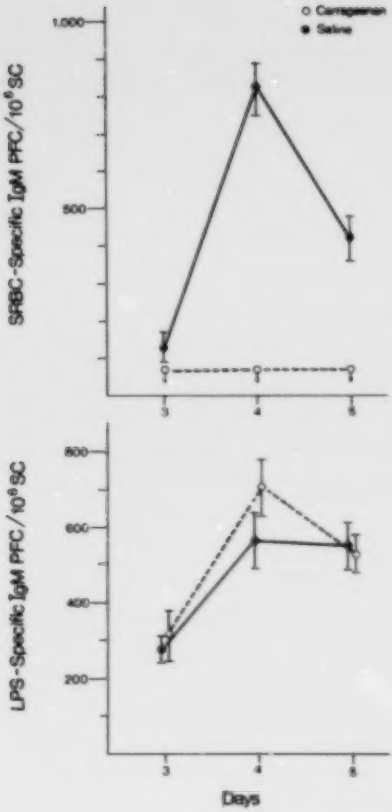

Figure 5. Effect of carrageenan on the PFC response to SRBC and LPS in vivo. Different groups of A/J mice were given two intraperitoneal injections of carrageenan, 0.5 mg per injection, 5 and 2 days before their immunizations with either 5 x 10^8 SRBC, a macrophage-dependent antigen, or 10 ug of E. coli LPS, a macrophage-independent antigen. Animals in each control group were similarly immunized but the pretreatment with carrageenan was replaced by the injection of saline. The SRBC-specific (upper panel) and LPS-specific (lower panel) PFC response of the injected animals was assayed 3 to 5 days after antigen challenge. Each point represents the mean PFC response ± SEM of 6 to 8 animals (From Shek & Lukovich, 1982).

TABLE IV

IN VIVO SUPPRESSION OF THE BSA-SPECIFIC PFC RESPONSE BY THE ADMINISTRATION OF CARRAGEENAN IN A/J MICE

Treatment[a]		Antigen	BSA-specific indirect PFC/spleen		
Day -5	Day -2	Day 0	Day 6		p[d]
Saline	Saline	LSM-BSA[b]	6,040 ± 360	(6)[c]	
Carrageenan	carrageenan	LSM-BSA	84 ± 16	(6)	<0.0001

[a] Animals were injected intraperitoneally with either saline or 0.5 mg of carrageenan each time on day -5 and day -2 before immunization.

[b] Immunized intraperitoneally with 50 µg LSM-BSA.

[c] Number of animals in each group.

[d] p value (Student's t-test) comparing the PFC response of the two groups.

eliminate or to minimize the induction of antibody formation against the membrane components.

So far, all studies investigating the efficacy of liposomes as an immunopotentiating carrier have been conducted by parenteral immunizations. The potential in the application of liposomal vesicles as a vaccine carrier for oral immunization has not been explored. In regard to this approach, it has been reported that oral administration of liposome-encapsulated biological compounds such as insulin (Patel & Ryman, 1976) and factor VIII (Henker et al., 1980) resulted in the absorption, though in limited amounts, of the active compound from the gastrointestinal tract into the circulation. The use of liposomes as a vaccine carrier for oral immunization will most likely promote IgA immunity. However, if sufficient entrapped antigenic materials find their way to non-gut-associated lymphoid organs, e.g., the spleen, via the lymphatics and the blood stream, the formation of antibodies of IgM and IgG classes may also be enhanced. The development of a suitable liposomal vaccine carrier for oral immunization undoubtedly requires the engineering of a vesicle with appropriate physicochemical characteristics which favour immunopotentiation via the gastrointestinal tract.

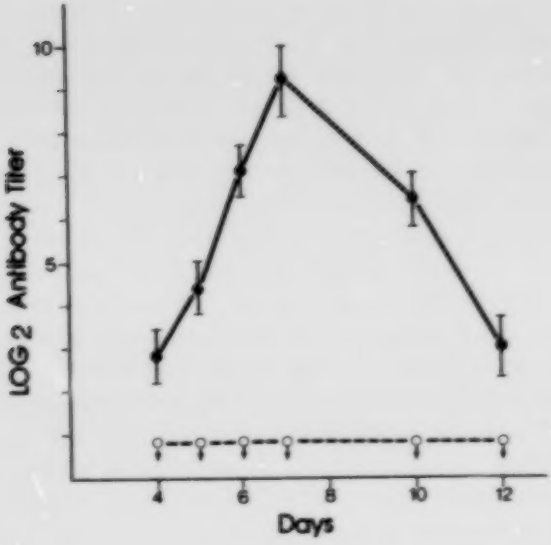

Figure 6. Suppression of circulating anti-BSA antibody titers by the administration of carrageenan in vivo. A/J mice in the experimental group (○) were injected intraperitoneally twice with carrageenan, 0.5 mg per injection, 5 and 2 days before their immunizations with 50 µg of LSM-BSA. Animals in the control group (●) were similarly immunized except the pretreatment was replaced with saline. The immunized animals were bled at different time intervals after immunization. Each point represents the mean serum anti-BSA hemagglutinating antibody titers of 6 animals. The inverted arrow indicates no detectable antibody titer.

POTENTIATION OF CELL-MEDIATED IMMUNITY

In addition to their ability to enhance antibody formation, liposomes are also effective in promoting cell-mediated immunity. Different types of cellular responses induced by liposome-associated macromolecules have been studied.

Proliferative Response

The proliferative response of cultured lymphocytes challenged with antigen is considered an in vitro correlate of cell-mediated immunity (Bloom, 1971). The

extent of cellular multiplication can be determined by measuring the incorporation of tritiated thymidine into DNA of the dividing cells. In an attempt to evaluate the efficacy of liposome-associated antigen in eliciting a primarily T-cell proliferative response, rabbits were immunized in one foot pad with liposome-encapsulated BSA and cells from the draining popliteal lymph node were subsequently cultured in the presence of the antigen. It is evident that lymph node cells, primed with liposome-entrapped BSA, elicited a significantly higher proliferative response than cells obtained from the control lymph node primed with free BSA (Fig. 7). It has been demonstrated that liposomes injected via the foot pad are localized predominantly in the regional lymph nodes (Ryman et al., 1978). This selective localization of the liposome-entrapped antigen may partly explain its apparently better efficacy in provoking a higher response. In contrast, the free antigen may channel through the lymph node more rapidly resulting in less antigen retention by the regional lymphoid cells. Since the T-cell proliferative response is facilitated by the participation of macrophages (Suzuki & Tomasi Jr, 1980), liposome-encapsulated antigen, being in particulate form, is more likely to enhance the uptake by phagocytic cells which in turn triggers an elevated T-cell response.

Induction of Cytotoxic T Lymphocytes

Liposomes have been shown to be effective carriers of major histocompatibility complex (MHC) antigens in inducing the formation of MHC-restricted cytotoxic T lymphocytes (CTL) in murine (Finberg et al., 1978) and human (Engelhard et al., 1978; Burakoff et al., 1980) systems. Previous attempts to investigate the molecular interactions in eliciting the CTL response were hampered by the necessity of using intact cells or cell membranes containing the stimulating antigens. Recent advances in liposome technology enable the incorporation of highly purified recognition molecules, e.g., MHC antigens, cell surface membrane components, and viral antigens in phospholipid vesicles that are capable of triggering the T-cell effector function. The presence of both viral and MHC antigens in liposomes is necessary for the successful stimulation of secondary CTL against specific viral targets (Finberg et al., 1978; Hale et al., 1980). The generation of the CTL response by liposome-incorporated MHC antigen requires the active participation of macrophages (Weinberger et al., 1980). Macrophages are involved in processing the liposome-associated antigen and in activating helper T cells which promote the CTL response (Weinberger et al., 1981).

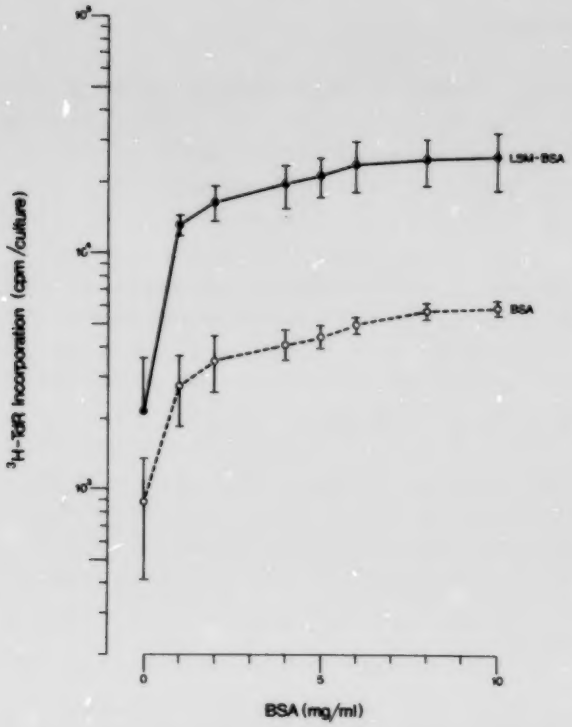

Figure 7. Potentiation of the proliferative response by liposome-associated BSA. Rabbits were immunized with 50 µg of LSM-BSA in one hind foot-pad, and 50 µg of free BSA in the other. Cells from each draining popliteal lymph node were assayed for their in vitro BSA-specific proliferative response 3 weeks after immunization. The total culture period was 72 hr and ^{3}H-TdR was added 18 hr before cell harvest. Each point represents the mean cpm ± SEM of 3 animals.

Macrophage Activation

Cell-mediated immunity may be expressed either by the direct action of the effector cell on the target, e.g., the lytic action on the viral target by CTL, or by the release of biologically-active soluble factors, called lymphokines, from activated lymphocytes which are predominantly T cells. One of the many lymphokines obtainable from lymphocytes stimulated by antigens or mitogens is the macrophage activating factor (MAF). Macrophages stimulated by the appropriate lymphokine are more active than resting macrophages in performing phagocytic functions. It is known that MAF-activated macrophages selectively destroy neoplastic, but not normal, cells (Fidler, 1978). The tumoricidal activity of macrophages activated by liposome-encapsulated MAF was much enhanced compared with the free factor (Poste et al., 1980). Using a tumor model in mice, it has been demonstrated that tumor metastasis can be eradicated by activating the tumoricidal functions of macrophages with liposome-encapsulated lymphokines (Fidler et al., 1980). These studies raise hope for the clinical application of liposome-encapsulated lymphokines in the treatment of metastatic tumors.

CONCLUDING REMARKS

In the preceding sections, the efficacy of liposomes in potentiating the humoral and cell-mediated immune response has been presented. It is clear that the versatility of phospholipid vesicles resides in the ease of altering their physicochemical characteristics for specific needs. The lipid composition and physical structure of a liposomal carrier can be adjusted to minimize its intrinsic immunogenicity yet retains its maximal adjuvant effect. Although the cell types involved in interacting with liposome-associated antigens and lymphokines are being identified, the basic mechanisms at the cellular and molecular levels mediating the potentiated response remain ill-defined. The elucidation of the relationship between the nature of liposome/antigen interactions and the potentiated immune response will facilitate a better design of vaccine carriers for human use. In view of the success, though limited, in using liposomal carriers for the delivery of labile substances via the gastrointestinal tract, the development of a suitable lipid vesicle for the delivery of oral vaccines may be on the horizon. Finally, the application of liposomal carriers for the delivery of other biological macromolecules must take into consideration the impact of potential immunological complications.

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